Strains of *Trichoderma* Benefit for Biological Control Seedlings Pathogens

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Introduction

During the next decade biological control may become an important component of plant disease management practices. The demand for alternatives to chemical control of plant pathogens has become stronger owing to concerns about the safety and environmental impacts of chemicals (4).

The most important pathogenic organisms in Siberia at forest nurseries are fungi which reside in soil and plant residues for extended periods. Among the main pthytopathogenic fungi are in genus *Fusarium*; species of *Alternaria* and *Pythium* are next in importance. *Trichoderma* species are economically benefit as examples of potentially useful biocontrol agents. The first requirement for successful biological control is selection of strains of an antagonistic microorganism. Screening strains can be conducted in four ways: 1) selection active strains in relation plant pathogens;

2) screening isolates which have high biotechnological indexes;

3) analyze of pathogens properties for plant, useful insects, animals and peoples;

4) search of substrates which cheapest and convenient for cultivation and saving of spores activity.

For purposes of developing effective biocontrol agent to combat damping-off in nurseries, we investigated 256 fungal strains in the genus *Trichoderma* which isolated in soil of Siberia.

Materials and methods

More 200 *Trichoderma* isolates were obtained from soil and organic compost and sawdust from various zones of Krasnoyarsk region. In tests during 1990 to 1997 were undertaken to evaluate competition, antibiosis and parasitism of each isolate against to phythopatogenes in genera of *Fusarium* and *Alternaria*.

Five of the most promising isolates were selected for inclusion in disease supressiveness tests. Initially, five strains were identified. Based on descriptions of Bissett (1,2,3) we classified this fungi as: 1. *Trichoderma* anamorph *Hypocrea gelatinosa* called MG; 2. *Trichoderma viride* called KR; 3. *Trichoderma virens* called O-97; 4. *Trichoderma longibrachiatum* called MK; 5. *Trichoderma harzianum* called U.

Isolates were screened for antagonistic activity toward the major damping-off pathogens of pine and picea seedlings in genus *Fusarium*. For comparison we used standard strain U (*Trichoderma harzianum*) which put into practice as biocontrol agent in Russia. We used the paper disk method of quantifying antagonistic activity (Yozef, 1983). Briefly, this procedure involved growing *T.species* in liquid Czapek medium for 15 days in sterile flasks (250ml). Fungal concentration was infested about 1×10^7 conidia/ml, after which the fungal mycelium was removed by passing the liquid through a membrane filter. This liquid extract was used to moisten sterile 5 mm paper disks. After moistering, the disks were dried and placed onto agar cultures of test pathogenic fungi. These plates were initially placed under refrigeration (10°C) for 10 hrs and then incubated at 28°C for 24 hrs. After incubation, width of the inhibition zones were measured. Strong inhibition was defined as greater than 30 mm; average inhibition from 15-20 mm, and weak inhibition below 15 mm. This experiment was replicated 5 times for each test antagonist with each pathogenic fungus.

Also, best biocontrol candidates were examined for biotechnological indexes: dry weight of mycelia and yield of conidia (5).

Next research was conducted comparative study of features of growth and sporulation on the various plant substrates obtained from a post extraction residuals of the picea and larix bark and solid residual of *Heliontus tuberosus* L. Substrates were placed into petri dishes at quantity 40 g/dish and infested spore suspension(1×10^6 conidia/ml). Isolates were incubated for 30 days at 28 °C.

Results and discussion

All tested strains in genus *Trichoderma* had high or moderate antagonist active toward *Fusarium* species. This research determined that maximum production of active metabolites were indicated on 15 day of growth isolates (Figure 1.) We also found that most resistant strain was *Fusarium* sporotrichiella to all *Trichoderma* species. Other necessary were indexes as well as production of conidia and amount of biomass. Results of this study shown at Figure 2. Comparisons of growth indicated that the strain O-97 was more amount of biomass than the another isolates but maximum of conidia had strains MG, MK and KR (Figure 3).

The ability to utilize plant substrates was basic and important for our researches. Our tests established that *Fusarium* species didn't colonize post extraction residuals of the picea and larix bark. However, solid residuals of Heliontus tuberosus was suitable for pathogens in genus *Fusarium*. Further details on the growth of *Trichoderma* species on various substrates are presented in Table 1. Substrate from solid residuals of *Heliontus tuberosus* was excellent for growth all *Trichoderma* species. We found that isolates may quickly colonize this substrate and utilize ligninlike substance and gemicellulosic. Post extraction residuals of the picea and larix bark were difficult mediums for all *Trichoderma* strains. Maximum yield of spores was revealed with isolates MK and U.

Conclusion

Our work has demonstrated that aborigine *Trichoderma* strains can be against towards phytopathogenes in genus *Fusarium*. From this experiments we concluded that selected isolates are potentially biocontrol agent as well as standard strain U. The ability of *Trichoderma* strains to utilize substrates from residuals of the picea and larix bark and residuals of *Heliontus tuberosus* may be lead to their commercial exploitation for creation cheapest biopreparations.

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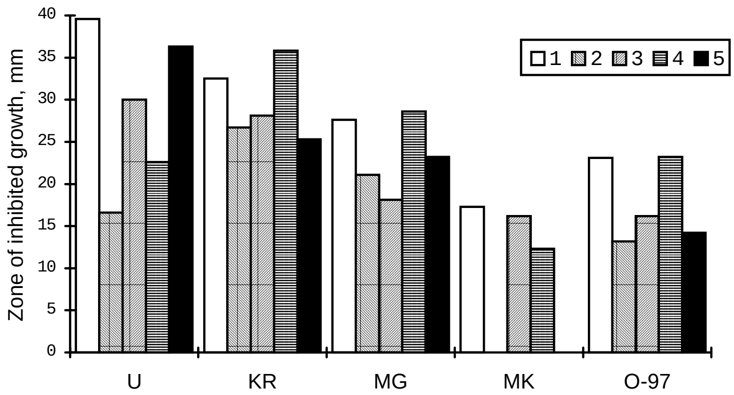
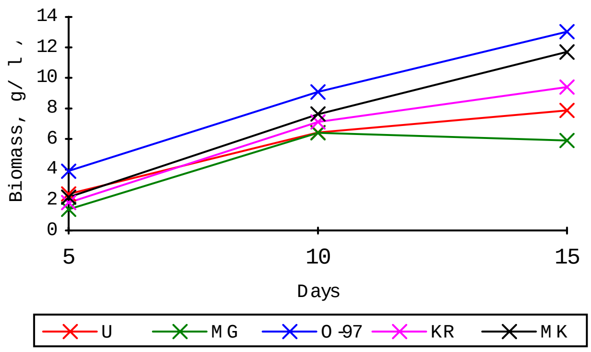


Figure 1. Antibiotic activity of selected strains of Trichoderma toward *Fusarium* species

1. Fusarium avenaceum,
2. Fusarium sporotrichiella v. poae,
3. Fusarium moniliforme v. lactis,
4. Fusarium nivale,
5. Fusarium gibbosum



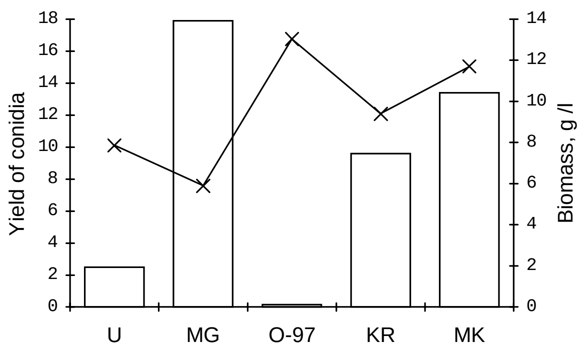


Figure 2. Biomass of selected strains of Trichoderma

Figure 3. Yield of conidia ($\times 10^{10}$ conidia/ L) and biomass (g / L) on 15th day of cultivation

Table 1

The yield of conidia of Trichoderma strains on plant substrates

Plant substrates	Yield of conidia, per L				
	Strains				
	U	MG	O-97	KR	MK
Post extraction residuals of the picea bark	$(1{,}00 \pm 0{,}08) \times 10^7$	$(7{,}10 \pm 0{,}09) \times 10^6$	$(3{,}37 \pm 0{,}22) \times 10^6$	$(8{,}60 \pm 0{,}04) \times 10^6$	$(1{,}00 \pm 0{,}06) \times 10^7$
Post extraction residuals of the larix bark	$(6{,}10 \pm 0{,}36) \times 10^6$	$(4{,}80 \pm 0{,}04) \times 10^6$	$(3{,}23 \pm 0{,}16) \times 10^6$	$(8{,}11 \pm 0{,}08) \times 10^6$	$(7{,}21 \pm 0{,}86) \times 10^6$
Residuals of *Heliontus tuberosus*	$(8{,}39 \pm 1{,}32) \times 10^8$	$(3{,}46 \pm 0{,}57) \times 10^8$	$(4{,}68 \pm 0{,}53) \times 10^8$	$(14{,}10 \pm 0{,}36) \times 10^8$	$(4{,}12 \pm 0{,}67) \times 10^8$